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Evaluation of Y-27632, a Rho-kinase inhibitor, as a bronchodilator in guinea pigs

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Abstract

To evaluate (+)-(*R*)-*trans*-4-(l-Aminoethyl)-*N*-(4-pyridyl) cyclohexanecarboxamide dihydrochloride, monohydrate (Y-27632), a selective Rho-kinase inhibitor, as a novel bronchodilator in vivo and in vitro, we investigated the effect of Y-27632 on the acetylcholine- or ovalbumin-induced increase in lung resistance (R_L) in non-sensitized or passively sensitized guinea pigs, and the relaxant effects of salbutamol, Y-27632 and theophylline on acetylcholine- or ovalbumin-induced contraction of isolated trachea. Y-27632 inhalation (1 mM, 2 min) inhibited acetylcholine- or ovalbumin-induced increase in R_L without changes in mean blood pressure, and the effect persisted for at least 3 h. Salbutamol, Y-27632 and theophylline each completely reversed the acetylcholine- or ovalbumin-induced contraction of isolated trachea with rank order of potency, salbutamol > Y-27632 > theophylline. The relaxant effect of Y-27632 was not affected by propranolol. We conclude that, although Y-27632 is not as potent as a β-adrenoceptor agonist, Y-27632 may become an alternative inhaled bronchodilator, because Y-27632 is more potent than theophylline, and the relaxant effect is independent of β-adrenoceptors.

Keywords: Bronchodilator; Rho-kinase; Y-27632

1. Introduction

A small G protein, RhoAp21, and its target protein, Rho-kinase, contribute to Ca^{2+} sensitization of contraction in a variety of smooth muscles (Gong et al., 1996; Hirata et al., 1992; Somlyo and Somlyo, 1994; Uehata et al., 1997). A highly selective Rho-kinase inhibitor, (+)-(*R*)-*trans*-4-(l-Aminoethyl)-*N*-(4-pyridyl) cyclohexanecarboxamide dihydrochloride, monohydrate (Y-27632), reversed G protein-mediated Ca^{2+} sensitization in permeabilized mesenteric arteries, and Y-27632 decreased mean blood pressure in hypertensive rat models (Uehata et al., 1997). Similarly, receptor-dependent, G-protein-mediated Ca^{2+} sensitization occurs in canine, rabbit, and human airway smooth muscle in vitro (Iizuka et al., 1997, 1999; Yoshii et al., 1999), and Y-27632 completely relaxes airway smooth muscle through inhibition of Ca^{2+} sensitization (Yoshii et al., 1999).

The Y-27632 effect is likely to be independent of contractile receptor agonists. Further, the Y-27632 effect is independent of 3-isobutyl-1-methylxanthine, a phosphodiesterase inhibitor (Yoshii et al., 1999). These results suggest that the site of Y-27632 action is distinct from those of conventional bronchodilators such as β-adrenoceptor agonists and xanthine derivatives. However, Y-27632 has not been fully characterized as a bronchodilator in vivo and in vitro.

To address this issue, first, we investigated whether Y-27632 inhalation may inhibit the acetylcholine- or antigen-induced increase in lung resistance (R_L) without cardiovascular side-effects. In some experiments, we measured plasma concentrations of Y-27632, which was given to normotensive guinea pigs either intravenously (i.v.) or by inhalation. Second, we compared the relaxing potency

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of Y-27632 with that of conventional bronchodilators, salbutamol and theophylline, in isolated trachea contracted with acetylcholine or antigen.

2. Materials and methods

2.1. Animals

Pathogen-free male Dunkin Hartley guinea pigs (Imai Dobutsu, Saitama, Japan) (450–550 g) were used. All procedures were in accordance with the recommendations of the council of Animal Care and Experimentation Committee, Gunma University, Showa (Iizuka et al., 1999; Uno et al., 1997; Yoshii et al., 1999).

2.2. Passive sensitization of guinea pigs

The animals were sensitized intravenously with 0.2 ml/kg of rabbit anti-ovalbumin antiserum (4 h heterologous passive cutaneous anaphylaxis titer > 2000), and used in the in vitro and in vivo experiments 24–48 h after sensitization (Dobashi et al., 1996).

2.3. Measurement of pulmonary function

The animals were anaesthetized with an intraperitoneal dose of urethane (1.5 g/kg), and a tracheal cannula was inserted via a tracheostomy and sutured in place. A catheter was inserted into the right carotid artery to monitor systemic mean blood pressure with a pressure transducer (COBE®, Lakewood, CO), and blood samples to measure Y-27632 concentrations were taken from a branch of this catheter (Uno et al., 1997). Another catheter was inserted into the left jugular vein for intravenous administration of Y-27632 and ovalbumin. All the animals were pretreated with propranolol (1 mg/kg, i.v.) 15 min before being challenged with acetylcholine or ovalbumin. Propranolol was used for inhibition of intrinsic catecholamine effects on airway (Uno et al., 1997). The animals, placed individually in a plethysmograph box with a pneumotachograph, were ventilated with room air through the tracheal cannula with a constant volume ventilator (Model 683, Harvard Apparatus, South Natick, MA) (Uno et al., 1997). The tidal volume was set at 10 ml/kg and the frequency to 60 breaths/min. R_L was determined by using a pulmonary mechanics analyzer (Model 6, Buxco Electronics, Sharon, CT), and was calculated from the airflow and ventilation pressures measured with transducers (type DP45-14 and type DP45-28, respectively; Validyne Engineering, Northridge, CA). R_L values before acetylcholine challenge were used as baseline levels in each animal. Aerosols were generated by an ultrasonic nebulizer (NE-U07, Omron, Tokyo, Japan) set at an air-flow rate of 1 ml/min (Uno et al., 1997). Under these conditions, small molecules (e.g. acetylcholine and Y-27632) but not relatively large proteins (e.g. ovalbumin) were constantly delivered to the bronchial tree. Therefore, to obtain reproducible results, ovalbumin challenge was performed intravenously. To study the dose–response relationship, saline (vehicle for Y-27632) or Y-27632 at the concentrations of 0.1, 0.3, 1 and 10 mM was inhaled for 2 min. Ten minutes after the pre-inhalation, acetylcholine at the concentrations of 0.1, 0.3 and 1 mM was inhaled for 1 min consecutively at 10 min intervals using the ultrasonic nebulizer. The intervals allowed a return to the pre-challenge baseline level. Hyperinflation with twice the tidal volume applied between each challenge was performed by manually blocking the outflow of the ventilator (Uno et al., 1997). Airway responsiveness to acetylcholine was expressed as the percent peak increase in R_L over the R_L and was calculated using the $-\log PC_{200}$ acetylcholine. This computation is a $-\log_{10}$ transformation of the concentrations of acetylcholine producing a 200% increase in R_L (Uno et al., 1997). To estimate the duration of the Y-27632 effect, a single dose of Y-27632 by inhalation (1 mM, 2 min) preceded the repetition of acetylcholine inhalation (0.3 mM, 1 min). Similarly, Y-27632 inhalation (1 mM, 2 min) was performed 10 min before ovalbumin challenge (1.0 mg/kg, i.v.) in passively sensitized guinea pigs, and R_L and mean blood pressure were monitored before and after ovalbumin challenge. In some experiments, to measure the systemic concentrations of Y-27632, 1 ml arterial blood was sampled when mean blood pressure reached a maximum low (approximately 6 min after the Y-27632 injection or inhalation). Serum was stored at −80°C until Y-27632 assay by high performance liquid chromatography (HPLC).

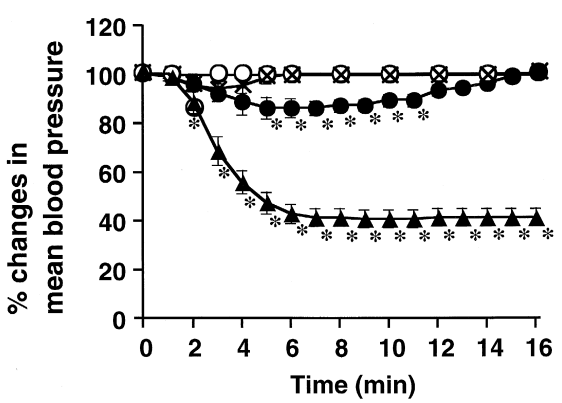

Fig. 1. Timecourse of changes in mean blood pressure after administration of Y-27632. During treatment with urethane and propranolol, the mean blood pressure of guinea pigs was monitored before and after administration of Y-27632; saline inhalation (open circle), 1 mM Y-27632 inhalation (cross symbol), 10 mM Y-27632 inhalation (closed circle) and 1 mg/kg intravenous injection (closed triangle). Statistical significance was determined by Student's *t*-test with the Bonferroni correction for multiple comparisons ($n = 4–6$). $^*P < 0.05$, saline inhalation vs. Y-27632 administration.

Table 1
Changes in mean blood pressure and concentration of Y-27632 after Y-27632 inhalation in guinea pigs

Dose of Y-27632	Body weight (g)	Initial mean blood pressure (mm Hg)	Percentage fall in mean blood pressure	Concentration of Y-27632 (ng/ml)	*n*
Saline	498.3 ± 13	44.2 ± 2.3	0.4 ± 0.4	under detection level	6
1 mM	492.7 ± 32	47.3 ± 2.0	8.3 ± 2.7	under detection level	6
10 mM	480.0 ± 18	52.3 ± 7.7	15.3 ± 4.8[a]	86.6 ± 11	5

Mean blood pressure was monitored before and after saline or Y-27632 inhalation for 2 min. The effect of Y-27632 on mean blood pressure is given as percent decrease in mean blood pressure (normalized to the initial mean blood pressure in each animal). Data are expressed as means ± S.E. and were compared by Student's *t*-test, using the Bonferroni correction for multiple comparisons.

[a] Indicates a P value < 0.05 considered to be significantly different from saline inhalation group. The number of animals is given as *n*.

2.4. HPLC assay of Y-27632 in plasma

A 100 μl of NaOH (1 M) and 2.0 ml of chloroform were added to 0.1 ml of plasma in a 12-ml glass-stoppered tube. The solution was shaken for 10 min and centrifuged for 5 min at 3000/min. The organic layer was evaporated at 40°C, the residue was redissolved in 200 ml of mobile phase and 150 ml was injected into the Hitachi L-7000 series HPLC system (Hitachi, Tokyo, Japan). The separation was done on a reversed-phase column, a Capcell pak C_{18} UG-120 S-5 (150 × 4.6 mm I.D., Shiseido, Tokyo, Japan), heated to 40°C. The mobile phase was methanol −20 mM sodium perchlorate adjusted to pH 2.5 with perchloric acid (1:9, v/v). The samples were eluted at a constant flow-rate of 1.0 ml/min, and the UV detector was set at 270 nm. The upper and lower limits of quantitation of the assay were 5000 and 50 ng/ml, respectively.

2.5. Tension experiments with guinea pig isolated trachea

The animals were anaesthetized with pentobarbital sodium (0.5 g/kg, i.p.), and the thorax was opened to remove the trachea. Dissection of the tracheal strips and measurement of isometric force were as previously described (Dobashi et al., 1996; Yoshii et al., 1999). Briefly, the strips were mounted vertically in a 10-ml Magnus tubes filled with Tyrode's solution aerated with 5% CO_2 in O_2 at 37°C. Ibuprofen (2 μM), a cyclooxygenase inhibitor, was present throughout the experiments. To obtain reproducible contractile responses of the strips without changes in Ca^{2+} sensitivity, application of 78 mEq/L high-potassium and its washing was repeated twice, and then a 1-μM acetylcholine-induced contraction (approximately 50% force of the maximal contraction induced by 100 μM acetylcholine) was evoked. When the acetylcholine-induced contractions were stable, various concentrations of salbutamol, Y-27632 and theophylline were

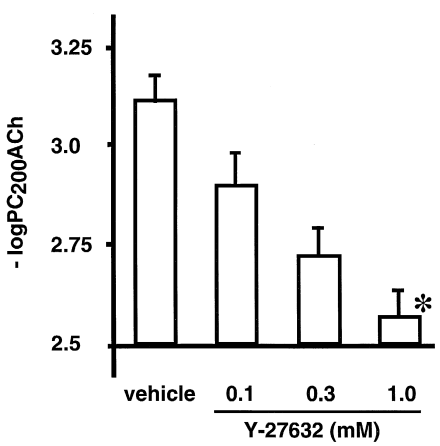

Fig. 2. Inhibition of acetylcholine-induced increase in lung resistance by Y-27632 inhalation. Ten minutes after inhalation of saline or Y-27632 (0.1–1 mM) for 2 min, acetylcholine at the concentrations of 0.1, 0.3 and 1 mM was inhaled for 1 min consecutively, with 10 min intervals, using an ultrasonic nebulizer. Airway responsiveness to acetylcholine was expressed as the percent peak increase in lung resistance (R_L) over the R_L, and was calculated by using the $-\log PC_{200}$ acetylcholine. This computation is the $-\log_{10}$ transformation of the concentrations of acetylcholine producing a 200% increase in R_L ($-\log PC_{200}$ ACh). Statistical significance was determined by Student's *t*-test with the Bonferroni correction for multiple comparisons ($n = 6$). $^{*}P < 0.05$, vehicle (saline) inhalation vs. Y-27632 inhalation.

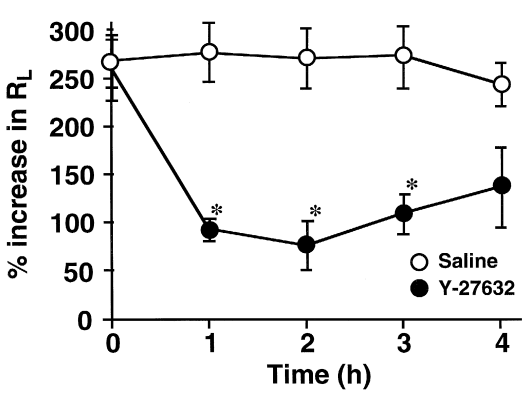

Fig. 3. Timecourse of inhaled Y-27632 effect. Ten minutes after inhalation of saline or Y-27632 for 2 min, a single dose of acetylcholine (0.3 mM) was repeatedly inhaled by guinea pigs for 1 min with 1 h intervals until the Y-27632 effect decreased ($n = 4$). The response to acetylcholine is expressed as the percentage increase in lung resistance (R_L) (normalized to the initial R_L in each animal). Statistical significance was determined by the Mann–Whitney *U*-test. $^{*}P < 0.05$.

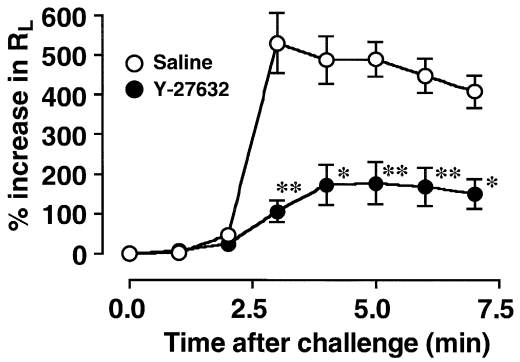

Fig. 4. Effect of Y-27632 inhalation on antigen-induced increase in lung resistance in passively sensitized guinea pigs. Ten minutes after inhalation of saline or Y-27632 for 2 min, ovalbumin (1.0 mg/kg) was intravenously injected to the passively sensitized guinea pigs. The response to antigen is expressed as the percentage increase in lung resistance (R_L) (normalized to the initial R_L in each animal). Statistical significance was determined by the Mann–Whitney *U*-test. $^{*}P < 0.05$, $^{**}P < 0.01$ ($n = 6$).

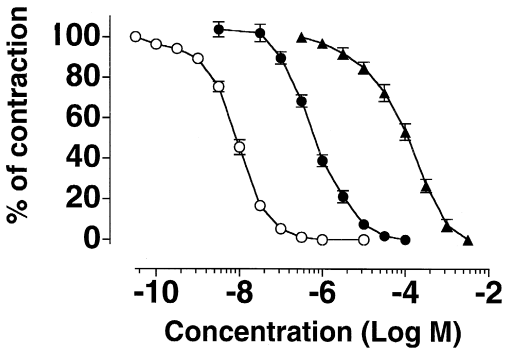

Fig. 5. Dose–response curves for salbutamol, Y-27632 and theophylline in acetylcholine-induced contraction of guinea pig isolated trachea. When acetylcholine (1 μM)-induced contraction of isolated trachea became stable, salbutamol (open circle), Y-27632 (closed circle), or theophylline (closed triangle) was cumulatively added to the bath ($n = 5$–6).

cumulatively added to the bath. To verify that the Y-27632 effect is independent of β-adrenoceptors, we tested relaxation of the acetylcholine-contracted trachea by Y-27632 in the presence of 3 μM propranolol. Similarly, when the ovalbumin (0.1 mg/ml)-induced force of the strips obtained from passively sensitized guinea pigs became stable, salbutamol, Y-27632 or theophylline was cumulatively added to each Magnus tube.

2.6. Reagents

Y-27632 was obtained from Yoshitomi Pharmaceutical (Osaka, Japan). Y-27632 was dissolved in saline as stock solution (10 mM), and stored at −20°C until use. Acetylcholine chloride, salbutamol hemisulfate, theophylline and ovalbumin were purchased from Sigma (St. Louis, MO). Propranolol hydrochloride was purchased from Sumitomo Pharmaceutical (Osaka, Japan). All other chemicals were of reagent grade.

2.7. Statistical analysis

The data as means ± S.E. were compared by the Mann–Whitney *U*-test or Student's *t*-test with the Bonferroni correction for multiple comparisons. A level of $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. Effect of Y-27632 on mean blood pressure

Under treatment with urethane and propranolol, Y-27632 injection (1 mg/kg, i.v.) to animals weighing 490 ± 12 g

Table 2
Absolute values in the experiments on antigen-induced of passively sensitized guinea pigs

Treatment	Body weight (g)	Initial R_L (cm H_2O/l/s)	Peak R_L (cm H_2O/l/s)	Initial mean blood pressure (mm Hg)	Min. mean blood pressure (mm Hg)	*n*
Saline	496.5 ± 28	211 ± 25	626 ± 167	49.5 ± 2.7	45.8 ± 2.9	6
Y-27632	499.2 ± 24	223 ± 17	1296 ± 95[a]	58.2 ± 3.4	46.7 ± 6.8	6

Lung resistance (R_L) and mean blood pressure were monitored before and after ovalbumin challenge (1.0 mg/kg, i.v.). Y-27632 inhalation (1 mM, 2 min) was performed 10 min before the challenge. Data are expressed as means ± S.E. and were compared by Mann–Whitney *U*-test.

[a] Indicates a *P* value < 0.01 considered to be significantly different from the control (saline inhalation) group. The number of animals is given as *n*.

Table 3
Comparison of the relaxant effects of salbutamol, Y-27632 and theophylline on acetylcholine-induced contraction of guinea pig trachea

Reagent	Acetylcholine-induced contraction (g)	IC_{50} (μM)	$-\log(IC_{50})$	n_H	*n*
Salbutamol	0.70 ± 0.07	0.0071 ± 0.0011	8.17 ± 0.08[a]	0.91 ± 0.02[a]	5
Y-27632	0.63 ± 0.11	0.69 ± 0.16	6.21 ± 0.10	1.56 ± 0.03	6
Theophylline	0.62 ± 0.11	84.1 ± 18.9	4.12 ± 0.10[a]	0.93 ± 0.10[a]	5

The concentration of reagent required for half-maximum relaxation (IC_{50}), $-\log(IC_{50})$ and Hill coefficient (n_H) are shown. The number of experiments is indicated by *n*. The data given as means ± S.E. were compared by Student's *t*-test, using the Bonferroni correction for multiple comparisons; Y-27632 vs. salbutamol or theophylline.

[a] Indicates a *P* value < 0.05 considered to be significant in the Bonferroni method.

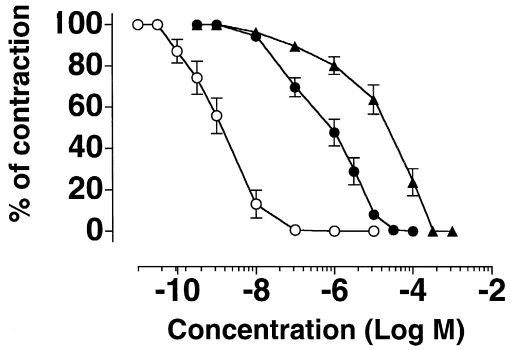

Fig. 6. Dose–response curves for salbutamol, Y-27632 and theophylline in antigen-induced contraction of guinea pig isolated trachea. When ovalbumin (0.1 mg/ml)-induced contraction of isolated trachea became stable, salbutamol (open circle), Y-27632 (closed circle), or theophylline (closed triangle) was cumulatively added to the bath ($n = 5$–12).

($n = 4$) decreased mean blood pressure from 44 ± 3 to 19.5 ± 3 mm Hg (approximately 56% decrease in mean blood pressure) within 6 min, and the effect was sustained for at least 30 min (Fig. 1). Higher doses of Y-27632 (3 and 10 mg/kg, i.v.) did not decrease mean blood pressure further (data not shown). The arterial concentrations of Y-27632 were 1506 ± 219 ng/ml ($n = 4$) 6 min after the Y-27632 injection (1 mg/kg, i.v.). Although Y-27632 at this dose inhibited the acetylcholine-induced increase in R_L, a fall in mean blood pressure was already observed at a 10 times lower dose of 0.1 mg/kg (data not shown). Thus, arterial tone was apparently more sensitive to systemic adeministration of Y-27632 than was bronchial tone under our experimental conditions. Next, we changed the drug delivery route for Y-27632 from intravenous injection to airway inhalation. As shown in Table 1, there was no difference in mean blood pressure values before Y-27632 application (initial mean blood pressure) for the control and Y-27632 inhalation groups. Although 10 mM Y-27632 inhalation transiently decreased mean blood pressure with detectable concentrations of Y-27632, 1 mM Y-27632 inhalation did not affect mean blood pressure. The time-course of changes in mean blood pressure after Y-27632 administration either by inhalation or by intravenous injection is shown in Fig. 1. Thus, at concentrations of 1 mM or less, Y-27632 inhalation did not affect the mean blood pressure values under our experimental conditions.

3.2. Inhibition by Y-27632 inhalation of acetylcholine- or antigen-induced increase in R_L

Y-27632 inhalation dose dependently inhibited the acetylcholine-induced increase in R_L. As shown in Fig. 2, 1 mM Y-27632 inhalation significantly decreased $-\log PC_{200}$ acetylcholine values from 3.07 ± 0.1 to 2.59 ± 0.1 ($P < 0.01$, $n = 6$). When 0.3 mM acetylcholine inhalation, which increased R_L approximately twofold, was repeated at 1 h intervals, the protective effect of Y-27632 was still observed at 3 h after the initial Y-27632 administration and repeated acetylcholine administration (Fig. 3). Next, we tested the effect of Y-27632 inhalation (1 mM, 2 min) on the ovalbumin-induced increase in R_L. As shown in Fig. 4, Y-27632 significantly inhibited the antigen-induced increase in R_L, although there was no difference in the mean blood pressure values between the control and Y-27632-inhaling groups (Table 2).

3.3. Relaxation of acetylcholine- or antigen-induced contraction of airway smooth muscle in vitro

As shown in Fig. 5, all the reagents completely relaxed the acetylcholine-contracted strips. Y-27632 was approximately 100 times less potent than salbutamol, but approximately 120 times more potent than theophylline (Table 3). Propranolol did not affect the relaxant effect of Y-27632; IC_{50} values for Y-27632 in the absence and presence of propranolol were 0.89 ± 0.12 and 0.66 ± 0.10 μM ($n = 6$). Antigen challenge caused a contraction that reached a peak within 10 min and was followed by gradual relaxation. When the force became stable, we added the relaxant agents cumulatively. Fig. 6 shows that salbutamol, Y-27632 and theophylline again relaxed the trachea with the same rank order of potency, salbutamol > Y-27632 > theophylline (Table 4). Thus, although Y-27632 relaxed the trachea completely, salbutamol was more potent than Y-27632 on both acetylcholine- and ovalbumin-induced contraction of airway smooth muscle in vitro.

Table 4
Comparison of the relaxant effects of salbutamol, Y-27632 and theophylline on antige-induced contraction of guinea pig trachea

Reagent	Ovalbumin-induced contraction (g)	IC_{50} (μM)	$-\log(IC_{50})$	n_H	n
Salbutamol	1.10 ± 0.3	0.0022 ± 0.0009	9.02 ± 0.25[a]	1.64 ± 0.67	6
Y-27632	0.79 ± 0.15	0.92 ± 0.26	6.41 ± 0.21	1.92 ± 0.24	12
Theophylline	0.93 ± 0.03	44.2 ± 10.8	4.41 ± 0.12[a]	1.15 ± 0.01	5

The concentration of reagent required for half-maximum relaxation (IC_{50}), negative logarithm of IC_{50} and Hill coefficient (n_H) are shown. The number of experiments is indicated by n. The data given as means ± S.E. were compared by Student's t-test, using the Bonferroni correction for multiple comparisons; Y-27632 vs. salbutamol or theophylline.

[a] Indicates a P value < 0.05 considered to be significant in the Bonferroni method.

4. Discussion

Y-27632 has an excellent selectivity to inhibit Rho-kinase as compared to other kinases, such as protein kinase C and myosin light-chain kinase in a cell-free system (Feng et al., 1999; Uehata et al., 1997). Thus, Y-27632 is a powerful tool to investigate the role of the Rho/Rho-kinase system, especially in vivo, although more potent Rho-kinase inhibitors (Tanaka et al., 1998; Uehata et al., 1997) have been synthesized.

Y-27632 inhalation effectively inhibited acetylcholine- and antigen-induced increase in R_L, and the Y-27632 effect was sustained at least for 3 h. The fact that augmentation of acetylcholine-induced Ca^{2+} sensitization occurs in a rat asthma model where Rho protein expression increased (Chiba et al., 1999) supports the idea that inhibition of the Rho/Rho-kinase pathway is suitable to reverse the augmented Ca^{2+} sensitivity of airway smooth muscle, especially the asthmatic phenotype.

In general, the important characteristic required for an inhaled bronchodilator is that it should produce few cardiovascular effects. In the original report (Uehata et al., 1997), Y-27632 decreased mean blood pressure in hypertensive but not in normotensive rats. Satoh et al. (1994) demonstrated that serotonin-induced Ca^{2+} sensitization was more prominent in coronary arteries of spontaneously hypertensive rats than in those of Wistar-Kyoto rats. These results suggest that Y-27632 may affect only the pathological part of smooth muscle hypercontraction. However, intravenous application of Y-27632 at 1 mg/kg, which is required for inhibition of the airway narrowing induced by acetylcholine inhalation, evoked a rapid and sustained decrease in mean blood pressure in normotensive animals. Ca^{2+} sensitization is a physiological phenomenon of smooth muscle contraction (Somlyo and Somlyo, 1994), and Y-27632 relaxed a variety of smooth muscles contracted in response to different types of contractile agonists with similar IC_{50} values (Uehata et al., 1997; Yoshii et al., 1999). The discrepancy of the Y-27632 effect on mean blood pressure between the original report (Uehata et al., 1997) and the present study may have the following reasons: (i) the treatment with urethane and propranolol inhibited feedback mechanisms to maintain mean blood pressure in vivo, (ii) intravenous injection of Y-27632 yielded much higher concentrations of Y-27632 than did oral application, (iii) the lower basal mean blood pressure of guinea pigs than of rats (approximately 50 vs. 100 mm Hg) allowed a marked response to Y-27632. Thus, systemic application of Y-27632 decreases the mean blood pressure of normotensive guinea pigs during treatment with urethane and propranolol.

Inhalation is expected to be a more suitable method for selective delivery of Y-27632 to the bronchial tree. Fortunately, Y-27632 is very soluble in water. Y-27632 inhalation at the concentrations of 1 mM or less effectively inhibited the acetylcholine response without apparent changes in mean blood pressure. This suggests that Y-27632 may become a new class of inhaled bronchodilator.

Y-27632 inhibited not only the acetylcholine but also the antigen-induced contraction both in vivo and in vitro. Several chemical mediators such as histamine, leukotrienes and platelet activating factor are involved in the antigen-induced airway smooth muscle contraction. Therefore, the Y-27632 effect seemed to be independent of the contractile stimulus.

Y-27632 was less potent than salbutamol, although it was more potent than theophylline. This indicates that Y-27632 is not likely to become a first line treatment to resolve air flow limitation. However, regular use of inhaled β-adrenoceptor agonists may cause deterioration of asthma control, and allergic reaction reduces β-adrenoceptor function (Sears et al., 1990). In such a situation, Y-27632 may become an alternative bronchodilator, because Y-27632 was more potent than theophylline, and the relaxant action of Y-27632 was observed even in the presence of propranolol both in vivo and in vitro.

In conclusion, although Y-27632 is not as potent as a β-adrenoceptor agonist, Y-27632 may become an alternative inhaled bronchodilator, because Y-27632 is more potent than theophylline and the relaxant effect is independent of β-adrenoceptors.

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